

Appendix S1.

PATIENTS AND METHODS

Study population

Individuals with AD (study group,  $n=281$ ) were recruited from the Department of Dermatology, Venereology and Allergy, Charité – Universitätsmedizin Berlin, Germany. AD was diagnosed by an experienced dermatologist according to published criteria (S1) with a mild–moderate (9–40) or severe (>40) phenotype, as defined by SCORAD (SCORingAtopicDermatitis) (S1). All patients with AD expressed elevated serum immunoglobulin E (IgE) concentrations. As a control group, 278 healthy, unrelated individuals, matched for age and sex before analysis were recruited by the Klinik für Dermatologie and the Institut für Mikrobiologie und Hygiene, Charité – Universitätsmedizin Berlin, Germany. Exclusion criteria for the control group were a positive individual or family history of AD, allergic asthma and allergic rhinoconjunctivitis. Table I summarizes the clinical data of both groups. All participants were adults (18–65 years) of Caucasian ethnicity. The study procedures followed were in accordance with the Declaration of Helsinki 1975, revised in 1983, and was approved by the ethics board of the Charité – Universitätsmedizin Berlin. Written informed consent was obtained from all participants.

Genotype assessment

DNA was isolated from ethylenediaminetetraacetic (EDTA) whole blood samples using the QiAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). *Cyp27b1* and *Cyp24a1*-SNPs were determined by real-time PCR with subsequent melting curve analysis employing a LightCycler480 (Roche Applied Science, Mannheim, Germany). Melting peaks were as follows: *Cyp27b1* rs703842. wt: 54°C and mut: 63°C; *Cyp27b1* rs10877012. wt: 54°C and mut: 59°C; *Cyp27b1* rs3782130. wt: 51°C and mut: 60; *Cyp27b1* rs4646536. wt: 53°C and mut: 63°C; *Cyp24a1* rs2248359. wt: 52°C and mut: 59°C; *Cyp24a1* rs2296241. wt:

50°C and mut: 57°C. Negative template controls were included in each reaction. The identified genotypes were validated from PCR products applying Sanger sequencing (data not shown). Details regarding the primer sequences and PCR conditions are shown in Table SI.

25(OH)D measurement

A single measurement of 25(OH)D serum concentration was obtained from 98 patients with AD and 45 healthy controls by using an enzyme linked immunosorbent assay (ELISA) (Immunodiagnosics Systems GmbH, Frankfurt/M, Germany). All sera were collected between October and March (low ultraviolet (UV) radiation). Serum 25(OH)D concentrations >75 nmol/l were regarded as sufficiency, 50–75 nmol/l as insufficiency and <50 nmol/l as deficiency (S2) (Table IV).

In silico analysis

The *Cyp27b1* and *Cyp24a1* genes were analysed as described previously in (S3) using UCSC genome browser (<http://genome.ucsc.edu/>) to identify the sequence of the human and mouse *Cyp27b1* and *Cyp24a1* gene including the flanking 5'-5000 bp upstream and 3'-1000 bp downstream. Evolutionary conservation was determined using mVISTA (<http://genome.lbl.gov/vista/mvista/submit.shtml>).

Statistical methods

Genotype frequencies were compared between non-atopic controls and patients with AD as such or stratified according disease severity. The allele frequencies were compared using  $2 \times 2 \chi^2$ -test and  $3 \times 2$  Cochran-Armitage trend test. Correction for multiple testing was performed by permutation analysis ( $10^6$  permutations; indicated as  $p_{\text{corr}}$ . Haploview software 4.2, [www.broadinstitute.org](http://www.broadinstitute.org) and (S4)). Whitney-Mann *U* test and Kruskal-Wallis test were used for independent groups (XL-STAT; [www.xlstat.com](http://www.xlstat.com)).  $p < 0.05$  was considered statistically

Table SI. Primer sequences and PCR conditions for genotyping Cyp27b1 and Cyp24a1

	rs703842 Annealing: 55°C, elongation time 13 s Melting point major=54°C; minor=63°C		rs10877012 Annealing: 56°C, elongation time 16 s Melting point major=54°C; minor=59°C
<i>Cyp27b1</i>	rs703842 F rs703842 R rs703842 S Anc rs703842	CAgCCTgCCTggTCACT CCCACCTCTTTAAgAgTACTgT TgCTCTTTCTCTAATCCCTggg gACgAggTCCAgCTAAgTAgAgTAAgCAg	rs10877012 F rs10877012 R rs10877012 S Anc rs10877012
	rs3782130 F rs3782130 R rs3782130 S Anc rs3782130	ggCCCTCTCTgTTgCCTAg CAACACTTTgggAggCTAAgg TTTTTTgTAgAgACAgTTCCACTATgT gCCCaggCTgTCTCAgACTC	rs4646536 F rs4646536 R rs4646536 S Anc rs4646536
	rs2248359 Annealing: 58°C, elongation time 11 s Melting point major=52°C; minor=59°C		rs2296241 Annealing: 54°C, elongation time 11 s Melting point major=50°C; minor=57°C
<i>Cyp24a1</i>	rs2248359 F rs2248359 R rs2248359 S Anc rs2248359	CCAACCTCCgTTCCTgAATTAgC ACCACgCCCCAgTAC gCCAgCCgCgATAACTCT AAATTgTgTATgTTgTCACTggACCTgCC	rs2296241 F rs2296241 R rs2296241 S Anc rs2296241
			gTTTTCTTCAACggCTTTAA AACgTggCCTCTTTCATCA CCCATAAAATCAgCCAAgACC TCAAAGAAAACAACgCAAAgACAC

All oligonucleotides in 5'-3' format. All PCR protocols included an initial 4 min 95°C denaturation step, 40 cycles (5 s 95°C, 20 s annealing temperature as indicated and elongation time as indicated by 72°C) followed by melting curve analysis. Melting point of the major and minor allele transcripts are provided in °C. F: forward sense; R: reverse primer; S: Sensor; 3'-FITC-labelled sensor probe; Anc: Anchor, 5'-LC640(Cy5)-labelled probe.

significant. The single SNP analysis, Hardy-Weinberg calculations, haplotype analysis and linkage analysis were performed by using the Haploview software.

#### SUPPLEMENTARY REFERENCES

- S1. Darsow U, Wollenberg A, Simon D, Taieb A, Werfel T, Oranje A, et al. ETFAD/EADV eczema task force 2009 position paper on diagnosis and treatment of atopic der-
- matitis. *J Eur Acad Dermatol Venereol* 2010; 24: 317–328.
- S2. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357: 266–281.
- S3. Heine G, Niesner U, Chang HD, Steinmeyer A, Zugel U, Zuberbier T, et al. 1,25-dihydroxyvitamin D(3) promotes IL-10 production in human B cells. *Eur J Immunol* 2008; 38: 2210–2218.
- S4. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21: 263–265.

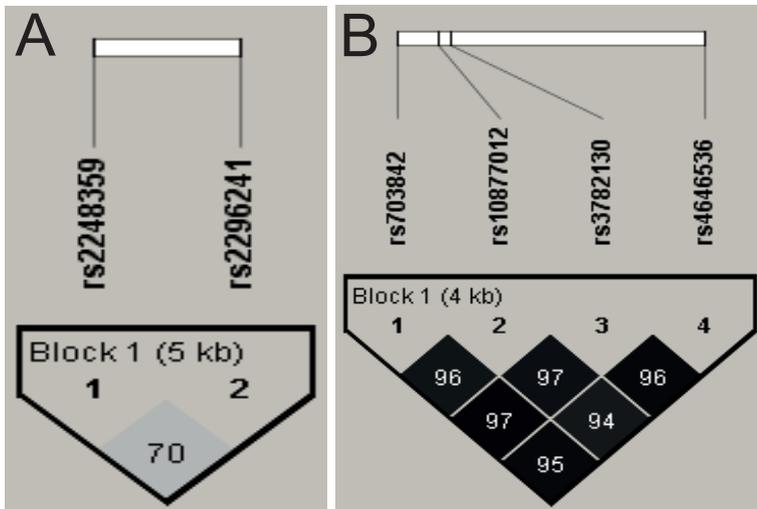


Fig. S1. Linkage disequilibrium analysis of the analysed genes in patients with AD and healthy controls. Data shown for (A) *Cyp24a1* and (B) *Cyp27b1* as  $D'$ -values (maximum linkage=100).

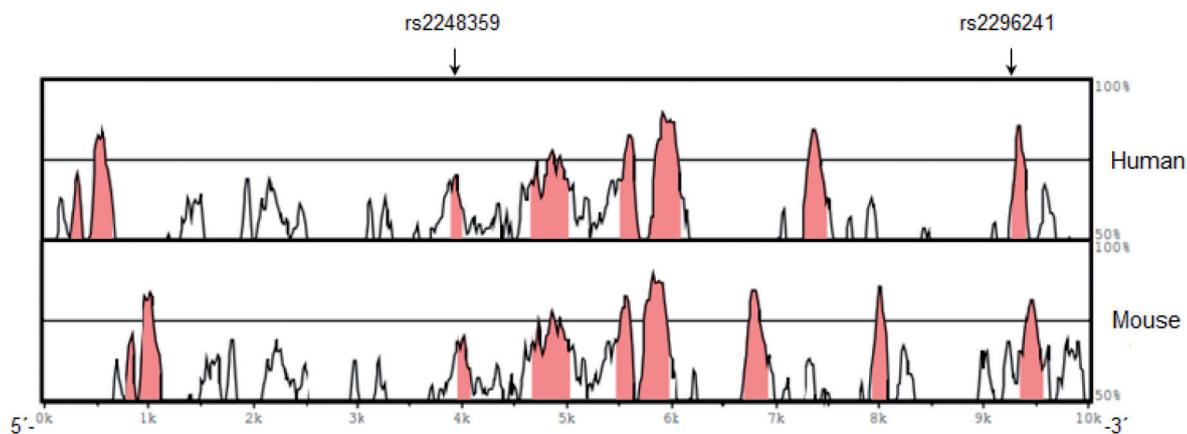


Fig. S2. Evolutionary conservation of the *Cyp24a1* gene. Analysis of the *Cyp24a1* gene including 5'-5000 bp flanking transcriptional start and 3'-1,000 bp were compared between human and mouse. The analysed SNPs are located as follows: rs2248359 (3,972 bp) and rs2296241 (9,271 bp). Pink areas indicate evolutionary conserved regions (>75% homology). Sequence displayed in 5'-3'-format.

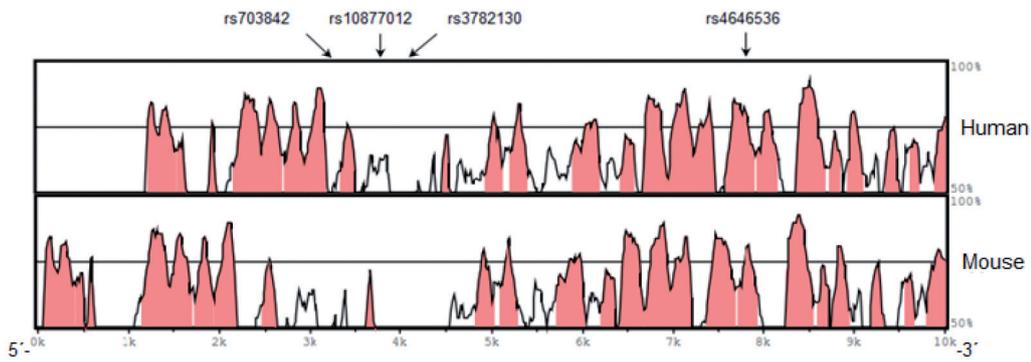


Fig. S3. Evolutionary conservation of the *Cyp27b1* gene. Analysis of the *Cyp27b1* gene including 5'-5000 bp flanking transcriptional start and 3'-1000 bp were compared between human and mouse. The analysed single nucleotide polymorphisms (SNPs) are located as follows: rs703842 (3211 bp), rs10877012 (3865 bp), rs3782130 (4052 bp) and rs4646536 (7962 bp). Pink areas indicate evolutionary conserved regions (>75% homology). Sequence displayed in 5'-3'-format.

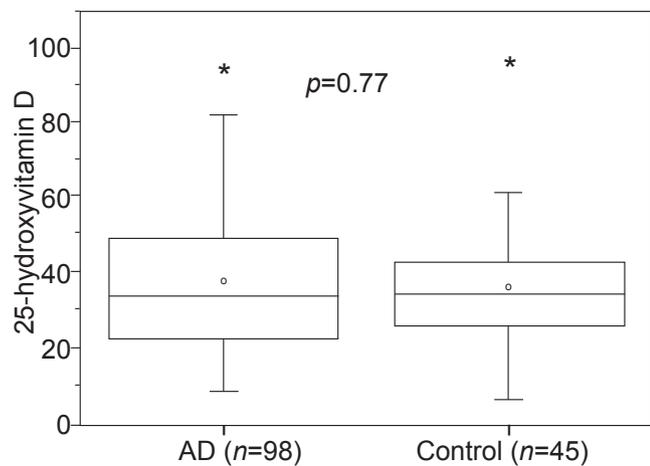


Fig. S4. Concentrations of 25(OH)D in the serum. The sera of patients with atopic dermatitis (AD) and healthy controls were obtained during the ultraviolet (UV)-deprived winter months, and concentrations of 25(OH)D were determined by enzyme-linked immunoassay (ELISA). Mann-Whitney *U* test was performed for statistical analysis.